

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 61-66 are in this case. Claims 62 and 64-66 have been rejected. Claim 63 has been objected to. Claims 64 and 65 have now been amended. Claim 66 has been canceled.

Drawings

The Examiner has rejected the petition for acceptance of color drawings (item 3 of detailed action) for lack of explanation of their necessity. Applicant wishes to point out that the color drawings of the instant specification depict the results of reduction to practice and proof of concept of the present invention, and as such are crucial to the instant specification. For example, Figure 5 depicts the effect of addition of biotin to T0 plants transformed with the constructs of the present invention. The instant specification describes the untreated (0d) plants as having "non-vital, chloridic leaves", while the treated plants (10d and 20d) are described as having "normal leaf development" (page 9, lines 13-22). Comparing the black and white and color versions of Figure 5, it is the Applicant's strong opinion that the distinctions indicated in the text (such as the "chloridic" character of the untreated leaves) cannot be appreciated from the black and white version.

Further, Figure 7, as described in the "Brief Description of the Drawings" section of the instant specification (page 10, lines 1-6), depicts the deleterious effects of streptavidin expression in tissues of transgenic plants of the present invention, and the restorative effects of biotin application. Comparing the black and white and color versions of Figure 7, it is the Applicant's strong opinion that the distinctions indicated in the text (such as the "severe morphological changes" observed in the untreated, transgenic plants) cannot be appreciated from the black and white version.

Applicant is confident that the abovementioned examples demonstrate the necessity of color drawings in the instant specification, and respectfully request withdrawal of the Examiner's rejection of the petition filed for their acceptance.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 62 and 64-66 under 35 U.S.C. § 112, first paragraph, stating that the specification does not provide reasonable enablement for one skilled in the relevant art to make and/or use the invention commensurate with the scope of these claims. The Examiner's rejections are respectfully traversed. Claims 64 and 65 have now been amended. Claim 66 has been cancelled, rendering moot the Examiner's rejection thereof.

The Examiner asserts that the specification does not reasonably provide enablement for methods of using streptavidin-encoding constructs with a secretion signal sequence and any tissue specific or developmental specific promoter to transform plants. Contrary to Examiner's assertion, it is Applicant's strong opinion that the instant specification provides sufficient enablement for one of ordinary skill in the art to transform plants with streptavidin-encoding constructs of the present invention in order to achieve expression and modification of specific plant tissue and/or developmental stages.

The present invention is of methods for effecting selective and reversible cell degeneration in plant tissue, and plants having controlled morphological changes resulting therefrom. While reducing the present invention to practice, the present inventors have shown that depletion of biotin in plant tissues by streptavidin-encoding constructs of the present invention effectively reduces the viability of specific plant tissues. Constitutive transient expression of the streptavidin in tomato plants, driven by the CaMV35S promoter, resulted in plants having extensive morphological and developmental deficiencies, from stems, to leaves to flower buds (see Table 3, page 47). Further, it was shown that tomato plants transformed with these streptavidin-encoding constructs also showed degeneration of tissues and loss of vitality (see Table 4 and Figure 5), with strikingly similar effects on canopy size, the effects of which could be reversed with external application of biotin (see Figures 5 and 7).

Further, the present inventors have shown that control of expression of streptavidin by the use of a tissue specific promoter results in degeneration of specific tissues. Expression of the streptavidin transgene under control of the Tob promoter resulted in defective fruit and seed development (see Figure 8). In similar

experiments, using the protocols taught in the present invention, as described in the Declaration attached herein, the present inventors have shown that expression in tomato of the streptavidin constructs taught in the instant specification, under control of the embryo and endosperm-specific French bean β -phaseolin promoter results in normal looking fruit having deformed and non-viable seeds (see Ginzberg et al, J Plant Physiol 2004; 161: 611-620; Figure 2b), attesting to the seed-specific expression of the transgene.

Applicant wishes to reiterate that the novel invention disclosed in the instant specification, and previously not taught in any prior art, is the reduction of available biotin in plant tissues by expression of streptavidin, and the ensuing, reversible cell degeneration. As detailed hereinabove, experimental results provide unequivocal proof of concept. Thus, one of ordinary skill in the art, privileged to the teachings of the instant specification, would be capable of constructing and using a streptavidin-encoding construct to transform plant tissue to express the streptavidin in specific tissues, and select viable plants exhibiting the desired characteristics, with a reasonable expectation of success. Tissue specific promoters are well known in the art, such as the French bean β -phaseolin promoter, the DLEC promoter, the PHS- β promoter, the zein storage promoter, and others recited in the instant specification (see, for example, page 26, lines 1-7). Additionally, plant-cycle-specific expression can be achieved by developmental-specific promoters such as the LEA and FbL2A promoters recited in the instant specification (page 26, lines 8-15). Other specific plant promoters have been employed in plant transgenic expression with great accuracy, for example the meristem and floral specific HMG2 Arabidopsis promoter (Hollick et al Plant Physiol. 1995;109:73-85) and the wound-specific win3.12 Populus promoter (Enjuto et al, Plant Cell 1995;7:517-27).

Applicant has nonetheless, in order to further define and clarify the claimed invention, elected to amend claim 64 to recite:

“A method of generating a plant having degenerated seeds comprising:

(a) expressing streptavidin regulated under the transcriptional control of a seed specific promoter in cells of a seed or seed-precursor tissue of a plurality of plants, said streptavidin including a signal peptide capable of self

secretion, thereby depleting biotin in said cells of said seed or seed-precursor tissue; and

(b) selecting viable plants of said plurality of plants which exhibit degeneration of said seed and/or seed precursor tissue as compared to similar plants not expressing said streptavidin, thereby obtaining the plant having degenerated and/or dysfunctional seeds.”

Support for such a limitation is found throughout the instant specification, for example, on page 39, line 19-26:

“According to the teachings of the present invention selective/controlled degeneration...of somatic plant tissue can be utilized to effect the following:... (iii) degenerate seeds...”.

Suitable seed-specific promoters disclosed in the instant specification include the DLEC promoter, soybean PHS β and conglutinin promoters, maize zein promoter, AT2S1 promoter, Arabidopsis ACT11 promoter, Brassica napus napA promoter, the LEA promoter and the tobacco prolamin promoter (see page 26, lines 1-15). These promoters are now recited in amended claim 65.

Thus, it is Applicant's strong opinion that the instant application teaches the use of tissue-specific expression of streptavidin under control of tissue-specific promoters which can be utilized for generating plants having degenerated tissue(s) (such as seeds) of the present invention.

Therefore it is respectfully submitted that claims 61-65 are now in condition for allowance. Prompt Notice of Allowance is respectfully and earnestly solicited.

Respectfully submitted,



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Registration No. 25,457

Date: April 20, 2004

Enclosed:

Two month's extension fee

Declaration and Curriculum Vitae of Dr. Yoram Kapulnik

Cited reference: Ginzberg et al